

FIG. 1A

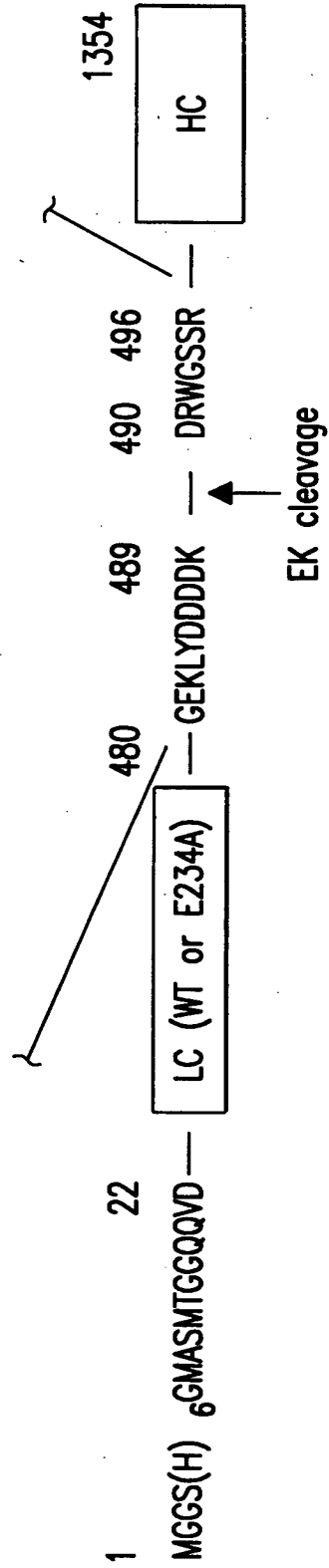


FIG.1B

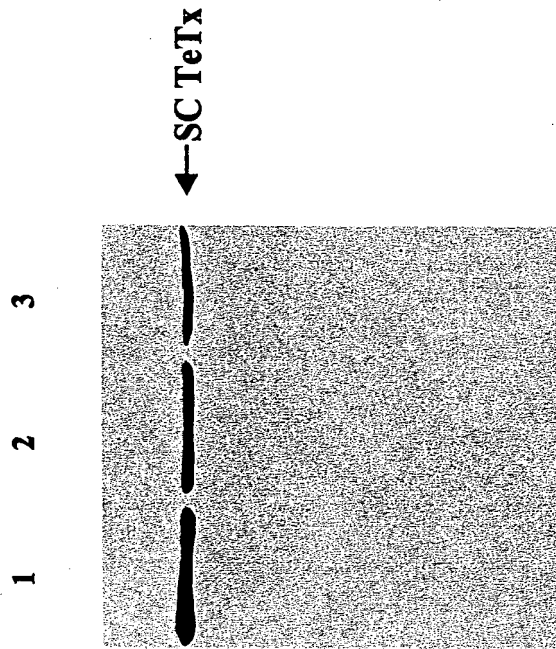
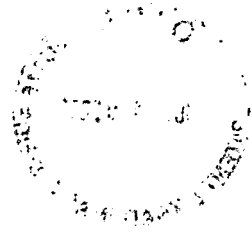


FIG.2B

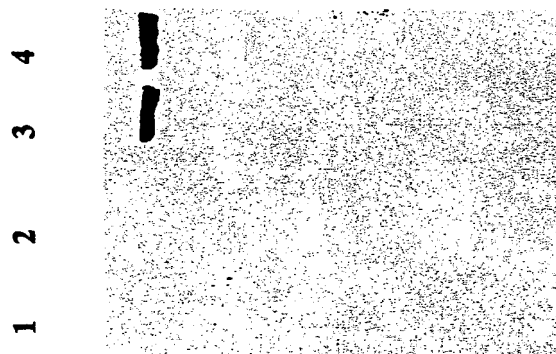


FIG.2A

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FIG.3A

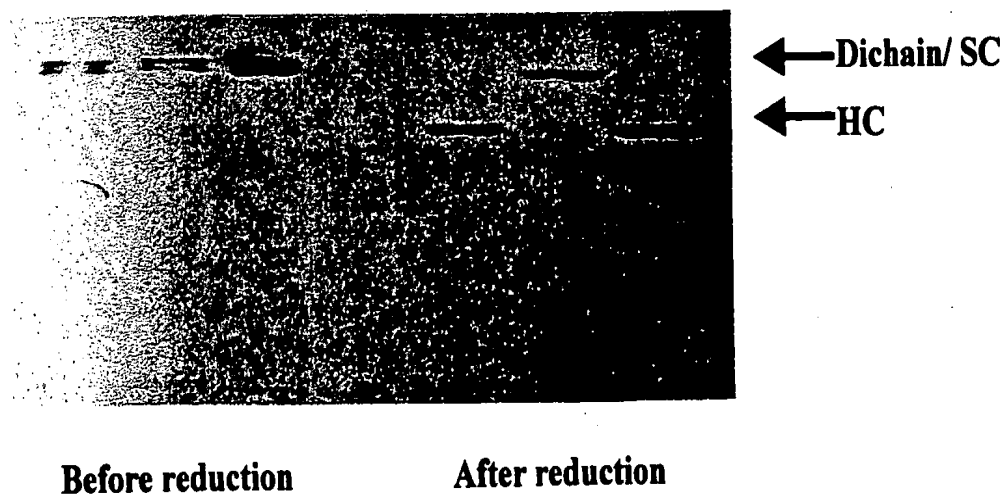
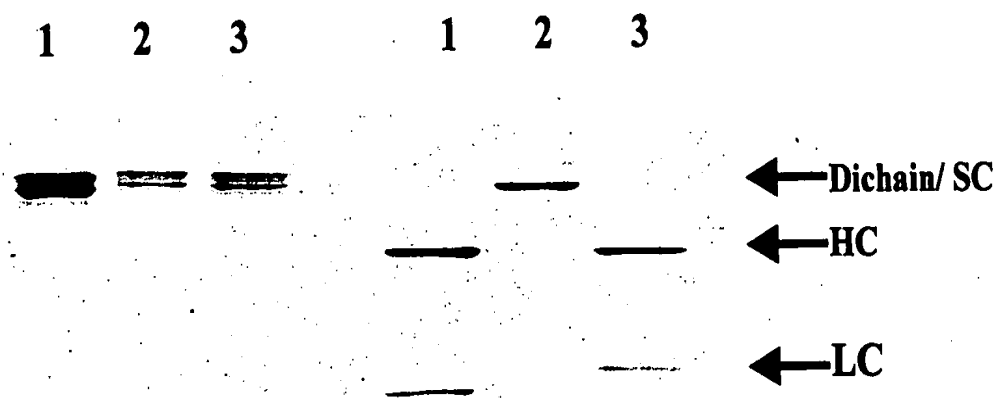


FIG 3B

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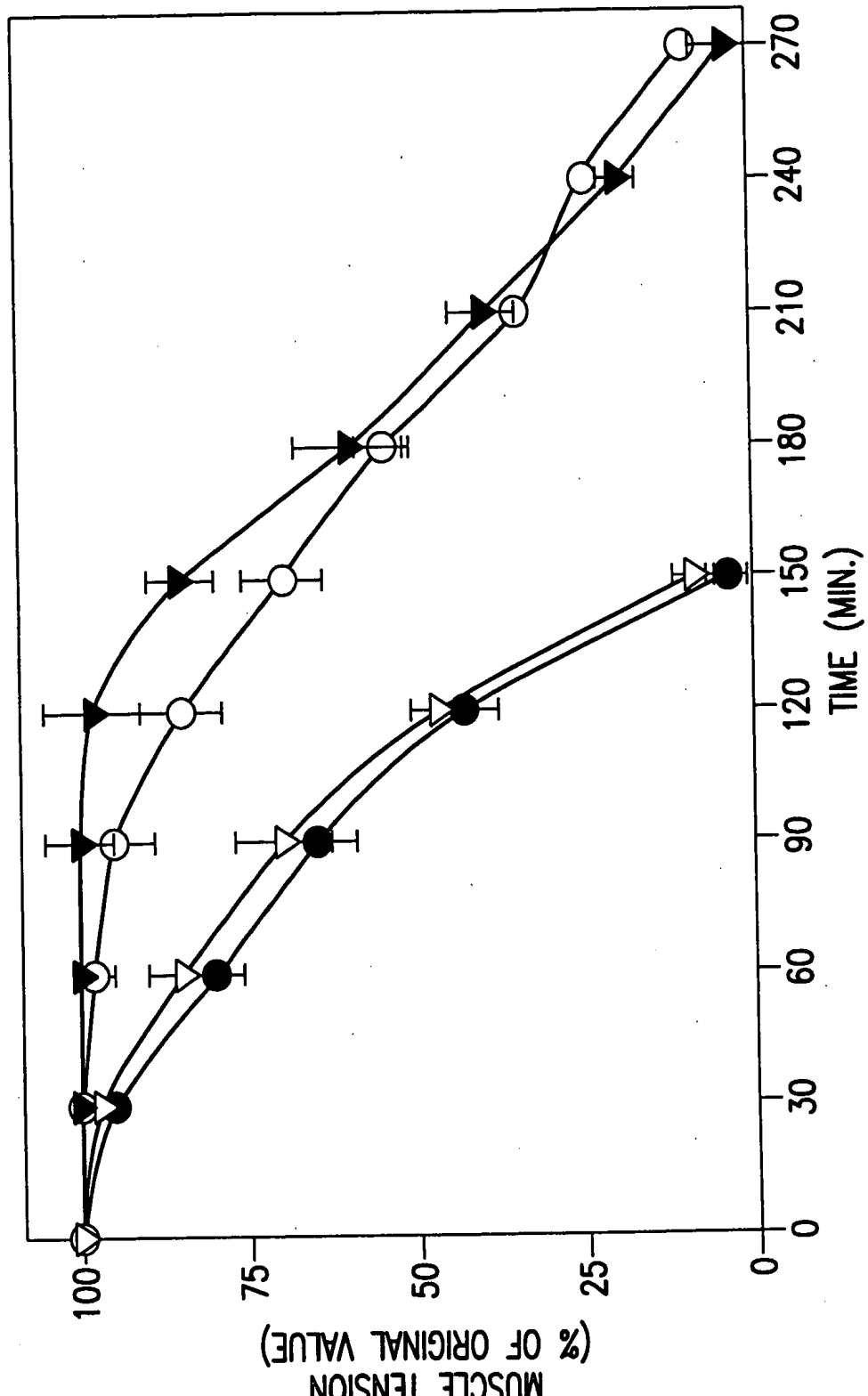


FIG. 4

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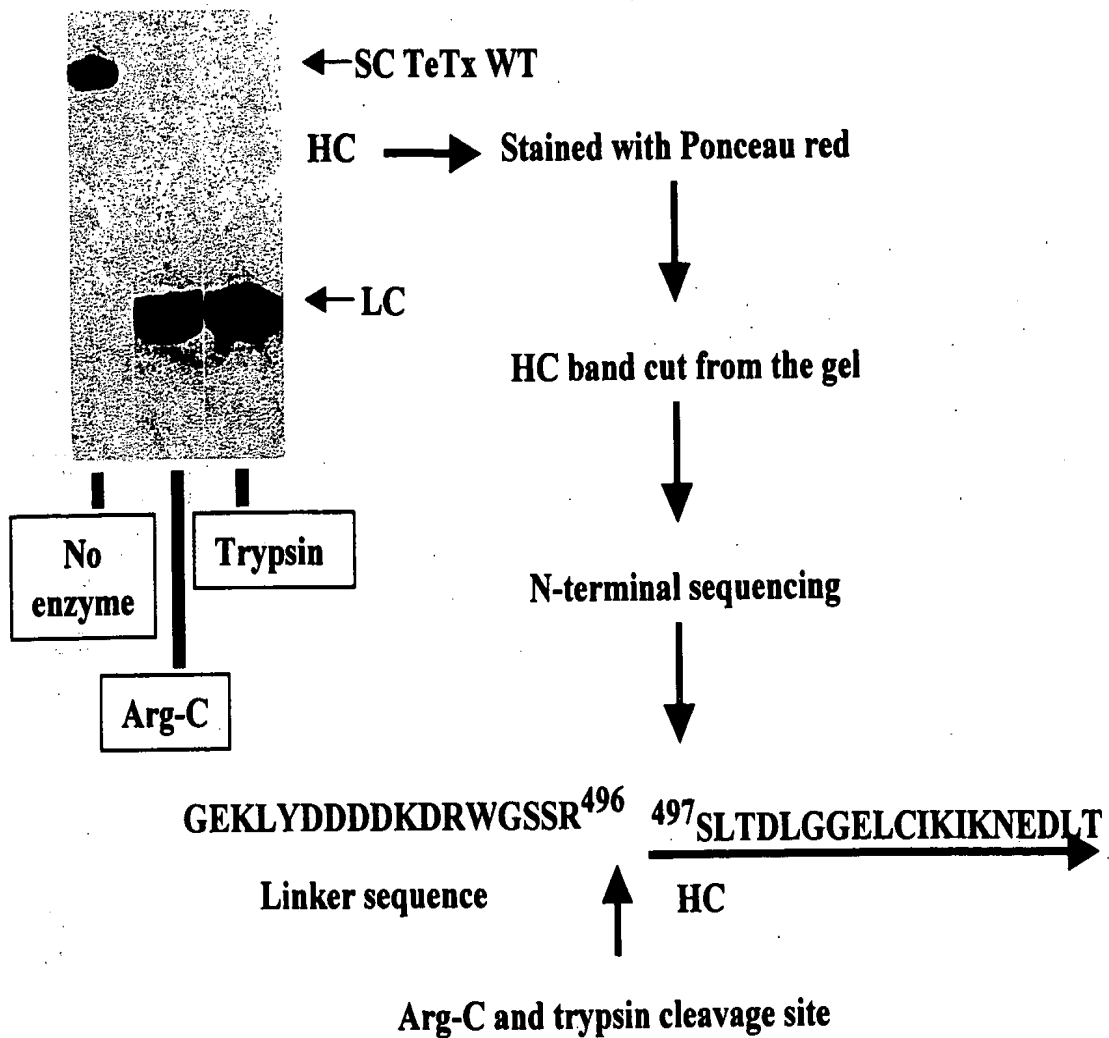


FIG 5

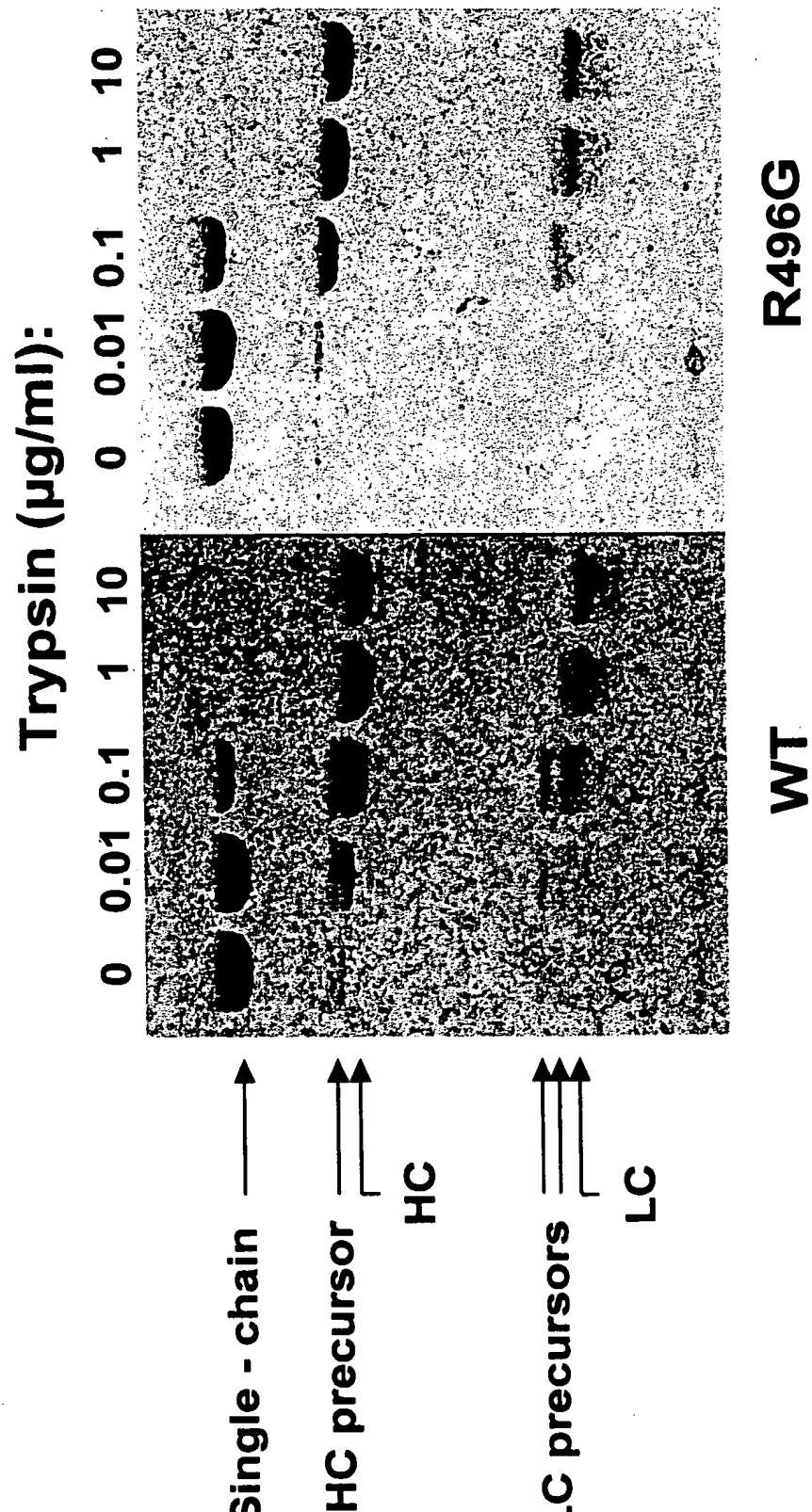


FIG.6

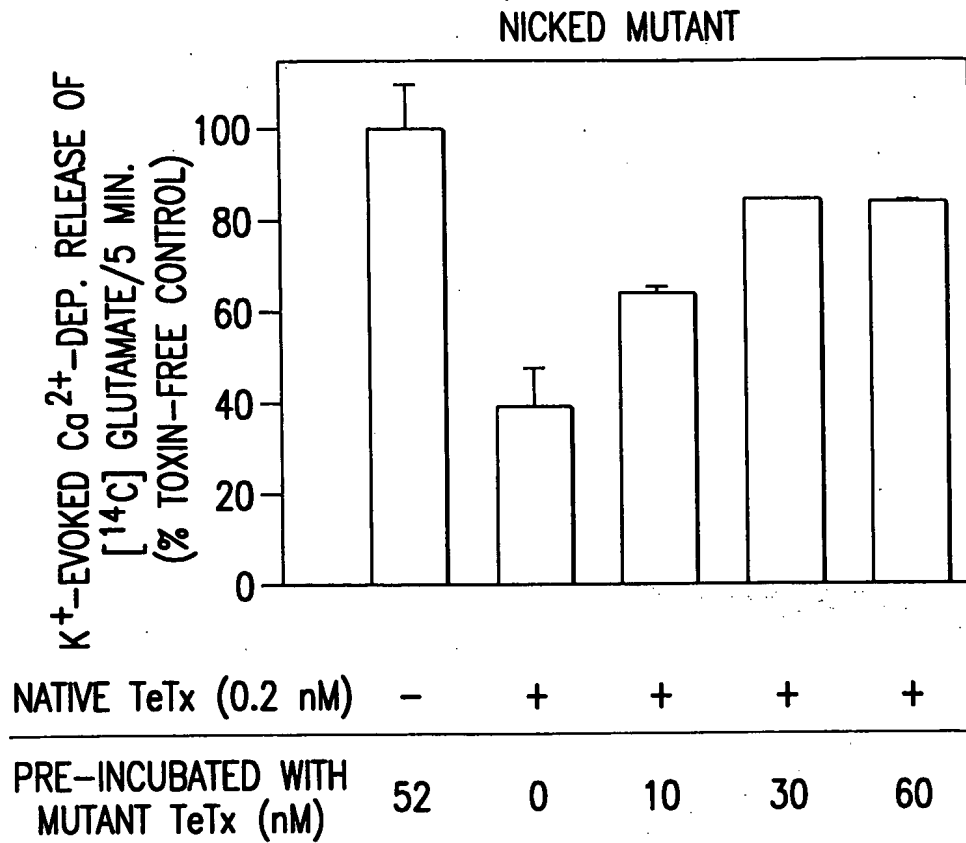


FIG.7A

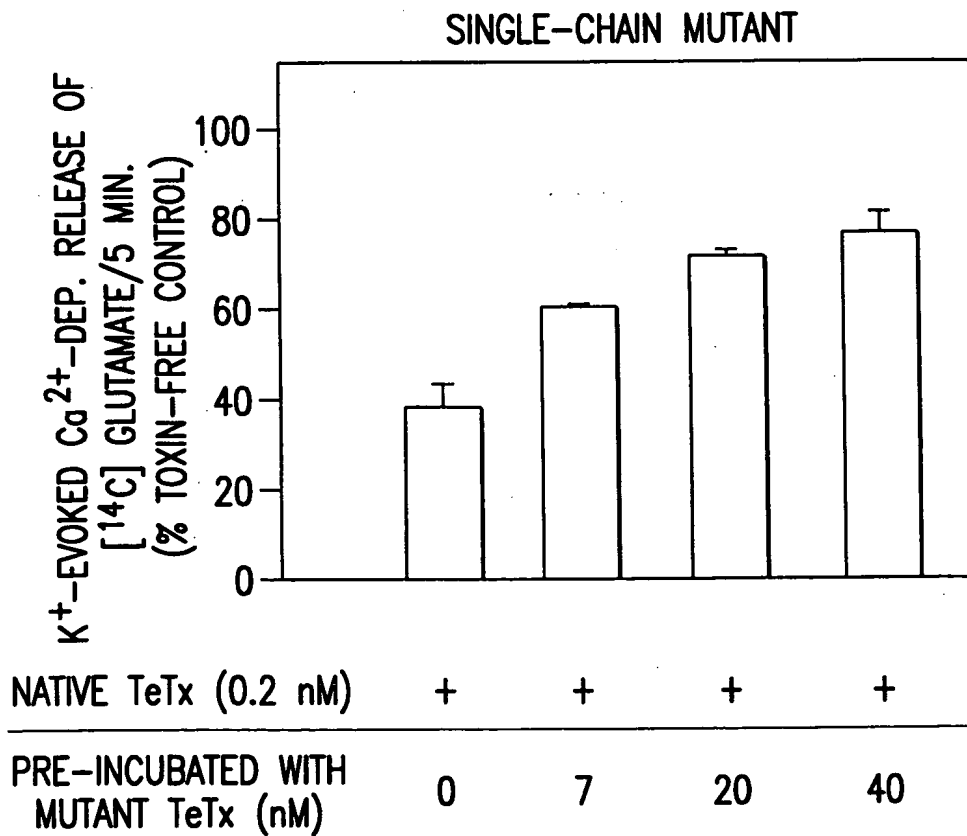


FIG 7B

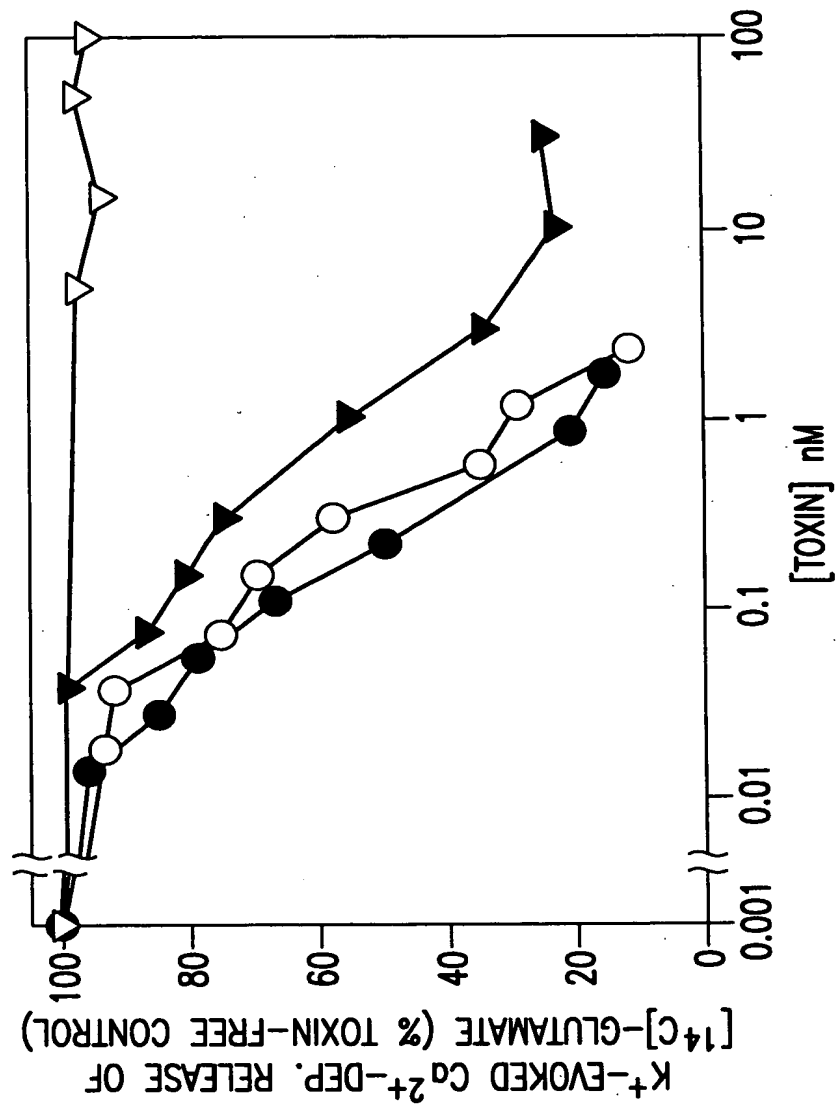


FIG.8

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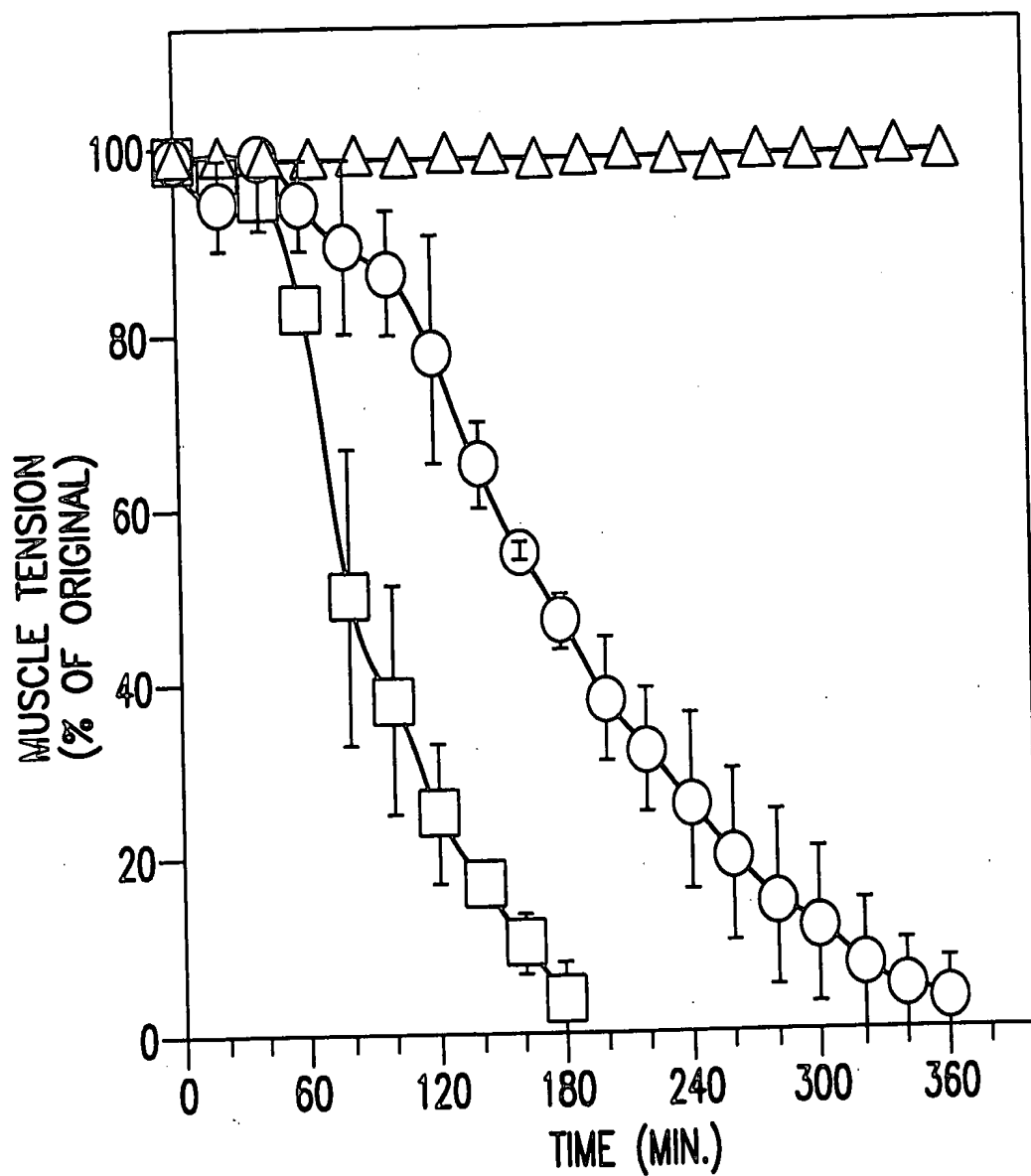


FIG 9

PCR WITH A PROOF READING
THERMOPOLYMERASE

1 2 3

1: MARKERS
2: NEGATIVE CONTROL
3: SAMPLE

3.5-kB DNA FRAGMENT PURIFIED AND CLONED INTO BamHI AND PstI SITES OF pQE30 EXPRESSION VECTOR

BamHI

HIS TAG

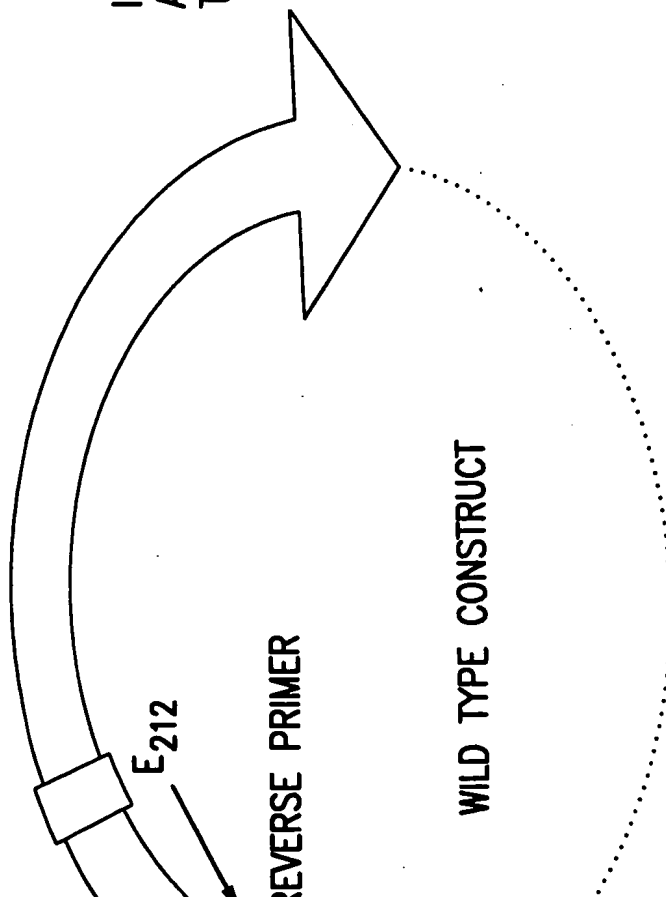
BoNT/E

PstI

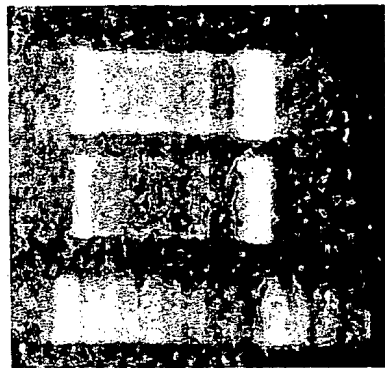
ATG AGA GGA TCG CAT CAC CAT CAC CAT CAC GGA TCC CCA AAA ATT AAT AGT TTT
M R G S H H H H H H G S P K I N S T
START POLY HISTIDINE TAG Bam HI BoNT/E LIGHT CHAIN

015

FORWARD PRIMER WITH E212Q SUBSTITUTION



INVERSE PCR WITH
A PROOF READING
THERMOPOLYMERASE



7-kb DNA FRAGMENT PURIFIED,
LIGATED AND TRANSFORMED IN *E.COLI*

E212Q SINGLE-CHAIN MUTANT

FIG.11

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CULTURE OF E.COLI M15[pQEESCwt]



OVERNIGHT INDUCTION WITH IPTG

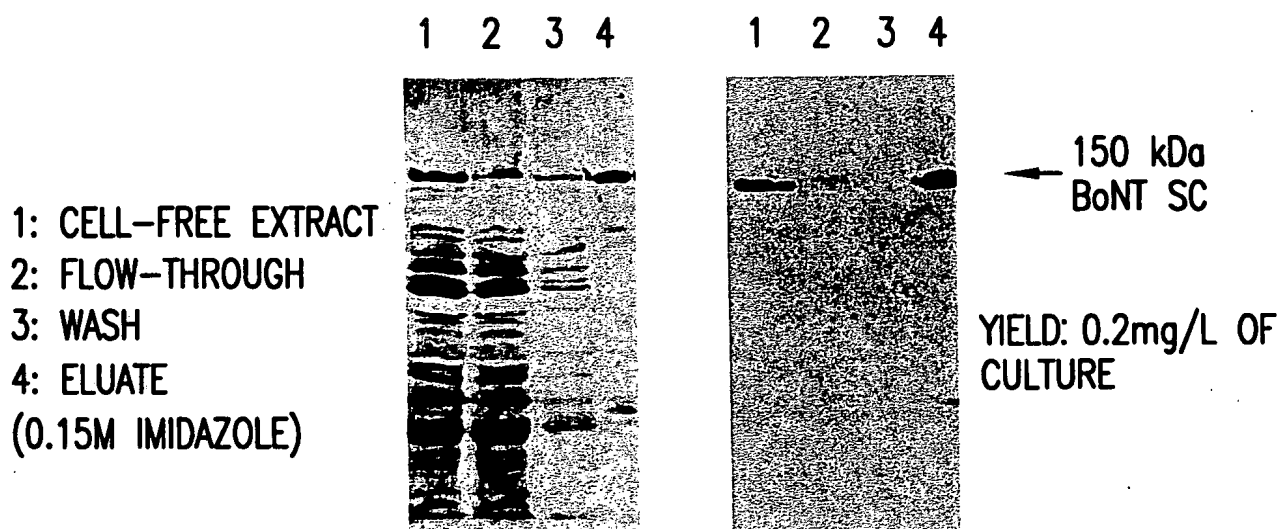


HARVEST, EXTRACTION AND CENTRIFUGATION



METAL CHELATE AFFINITY CHROMATOGRAPHY

SDS-PAGE (REDUCING COND.)

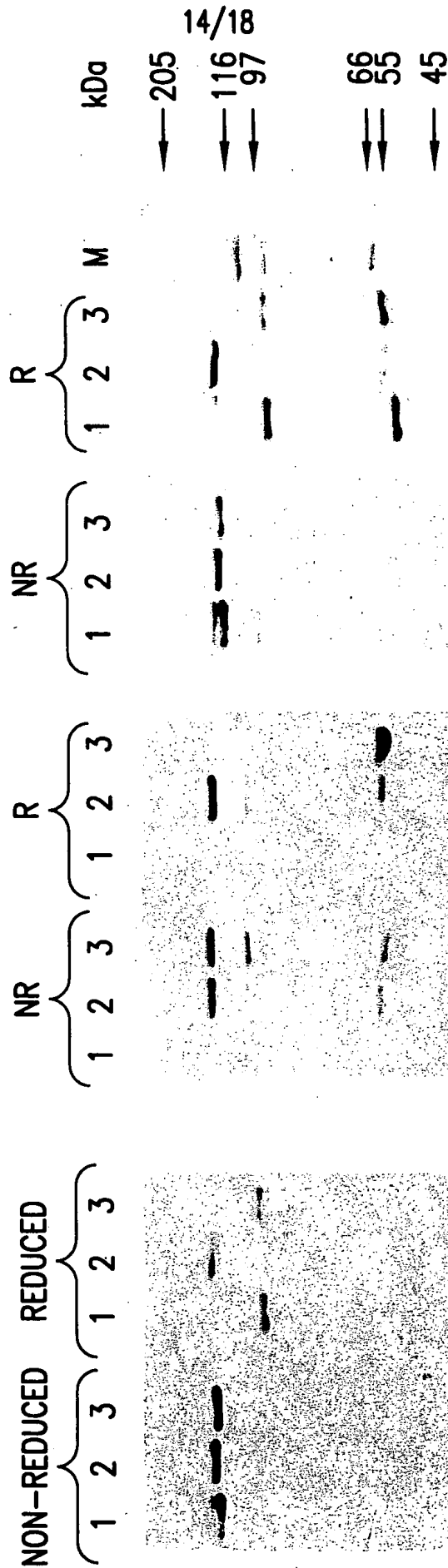


PROTEIN STAINED

WESTERN BLOTTED
WITH ANTI-HIS IgG

FIG.12

1: BoNT/E FROM *CLOSTRIDIUM BOTULINUM*
 2: UN-NICKED RECOMB. BoNT/E
 3: NICKED RECOMB. BoNT/E
 M: MARKERS



ANTI-Hc

ANTI-HIS

WESTERN BLOTTING

FIG.13

GST-SNAP-25(140-205)

INCUBATED WITH:

-DTT

+DTT

NATIVE DI-CHAIN

RECOMB. SINGLE-CHAIN

RECOMB. DI-CHAIN

(nM)

25

5

1

0.2

0.04

25

5

1

0.2

0.04

RECOMB. DI-CHAIN MUTANT

(nM)

0.2

1

5

25

125

0.2

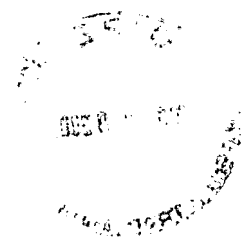
1

5

25

125

FIG.14



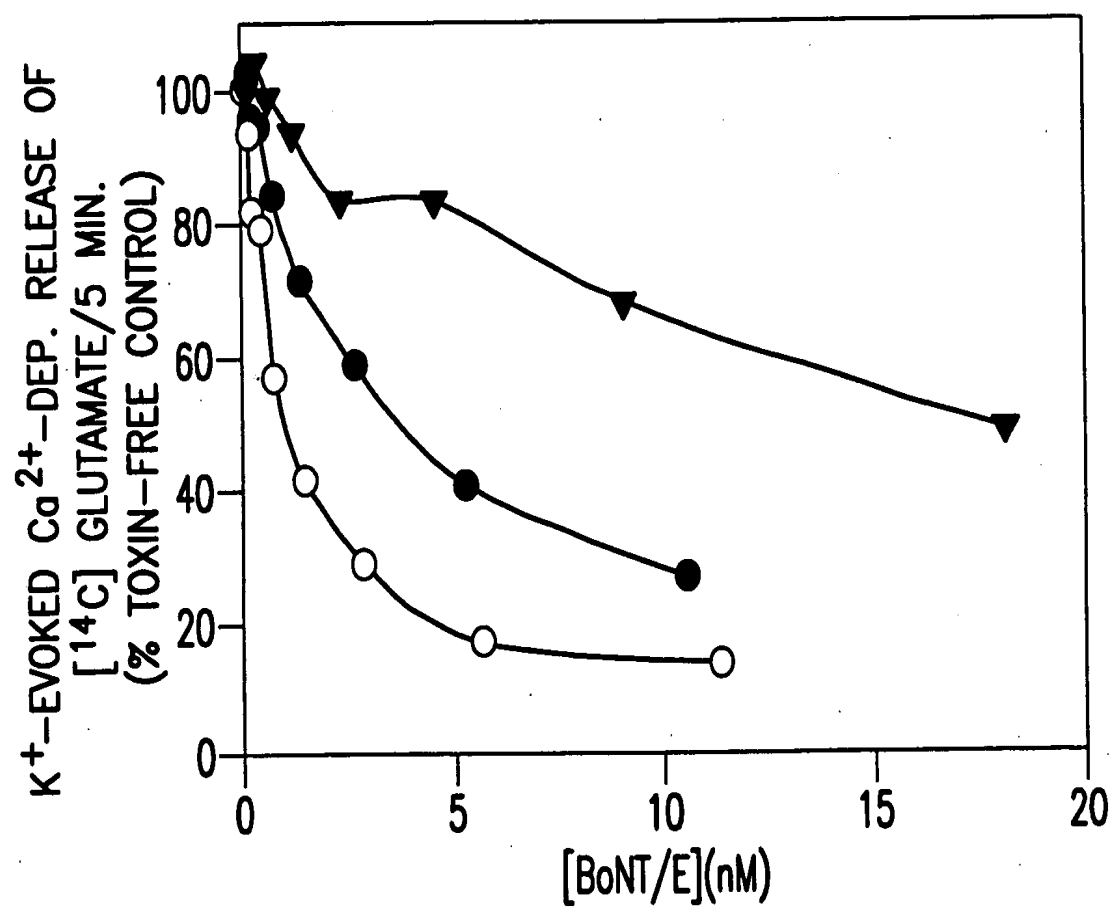


FIG. 15

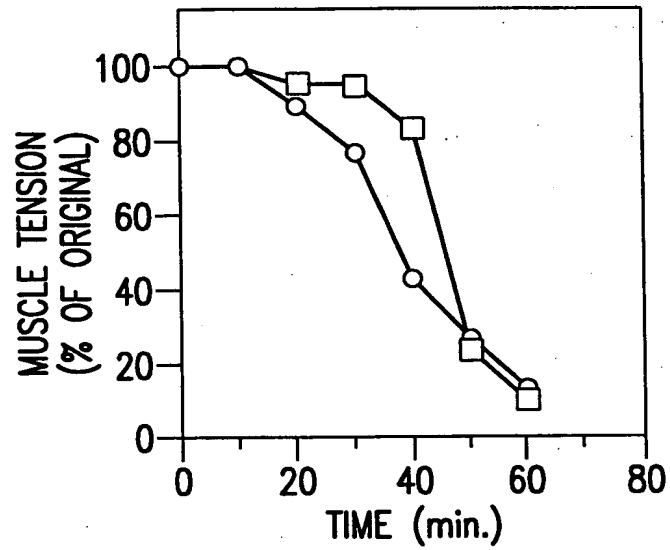


FIG. 16A

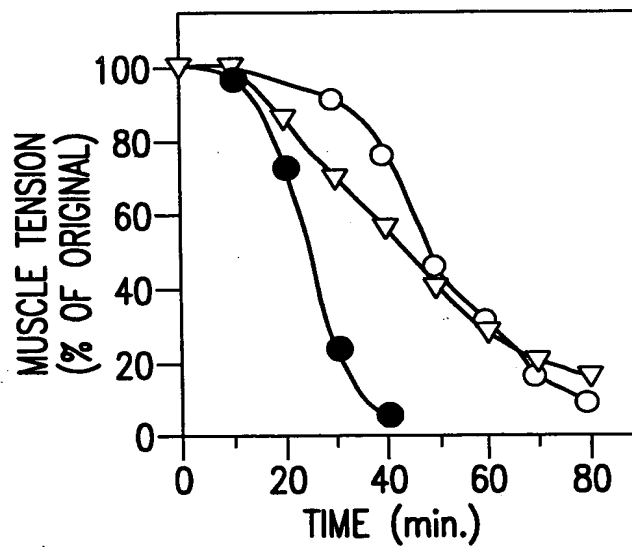


FIG. 16B

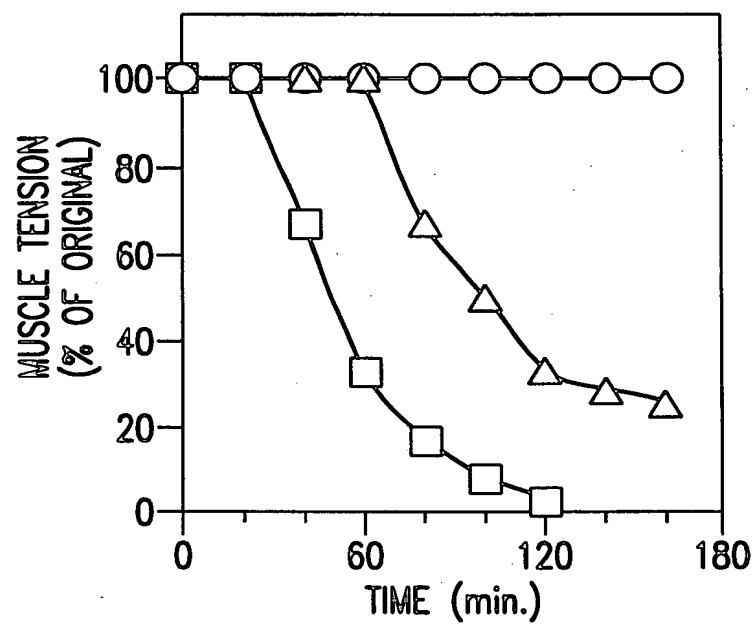


FIG. 17